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Rapid determination of pork sensory quality using Raman spectroscopy

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Rapid determination of pork sensory quality using Raman spectroscopy

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Prediction of Tenderness of Pork using Raman Spectrosensing

Abstract

Currently existing objective methods to evaluate tenderness and chewiness of pork in general do not yield satisfactory correlation to sensory panel evaluations, and their applications in meat industry are hence limited. In this study, a Raman spectrosensing method was developed to evaluate and predict tenderness and chewiness of pork loins. Raman spectroscopic binary barcodes for pork loins from 169 pigs were created based on their spectroscopic characteristics, and multivariate statistical discriminant model was developed based on the Raman barcodes to differentiate and classify pork loins into tenderness grades. Good agreement (> 82% correct predictions) with sensory panel results were obtained especially for pork loins that were at the extreme ends of tenderness (tenderness score < 8 or > 11) and chewiness (chewiness score < 2 and > 4) spectrum. The method developed in this report has the potential to become a rapid objective assay for tenderness and chewiness of pork products that may find practical applications in pork industry.

Key words: Raman spectroscopy, Pork Tenderness, Pork Chewiness, Partial Least Square, Support vector machine

INTRODUCTION

Fresh meat products in the market must look good to attract consumers; their physical appearance (i.e., color, texture, and lean to fat ratio) will be judged at the first glance. Moreover, attributes which could not be visually determined plays major roles in determining whether or not the consumer will buy the pork again(Munoz 1998). Tenderness and chewiness, which are among the major sensory attributes that dictates the joy for eating, are directly related to animal feeding practices, handling and the breeds of pigs. Evaluation of these sensory attributes may lead to prediction of customer responses to certain meat product, and bring great benefits to meat producers.

To this date, the best evaluation methods of sensory attributes which provide the most accurate prediction of customer responses are through sensory panels. The reason is obvious: panels are made up by human panelists whose evaluation best mimic general human responses. However, sensory panel evaluations are costly and time consuming. It is not possible to use it as routine quality assurance method in meat production. There is a great need for a rapid, non-destructive analysis technique that can be used to predict consumer responses.

Since tenderness and chewiness are primarily mechanical characteristics of the meat, a considerable number of studies have been conducted to investigate the correlations between physically measured mechanical properties (i.e., shear force, stress and strain response curves, etc.) with mixed results. Some reports showed strong correlations between mechanical properties (mainly Warner-Bratzler shear tests) of meat (i.e., beef) and the tenderness (Jeremiah and Phillips 2000), yet others suggested only weak correlations could be established (Chan, Walker et al. 2002). Due to this inconsistency, although mechanical properties measurement has the advantage of being objective, none of them has found wide application in meat production.

Both tenderness and chewiness of meat are dependent on three main factors: content and nature of connective tissue, sarcomere length and proteolysis (Koohmaraie and Geesink 2006). All these factors represent the biochemical characteristics of the muscle tissues. Rapid characterization of the overall biochemical landscape of the muscle tissues may provide indicators that can be used to construct prediction models that are better correlated to sensory panel evaluation results.

Vibrational spectroscopic techniques are the ideal tools to explore the general biochemical landscape of biological samples. Near infrared spectroscopy has been explored by many groups as a potential tool for providing an objective evaluation of meat sensory attributes (Mitsumoto, Maeda et al. 1991; Park, Chen et al. 1998; Rodbotten, Mevik et al. 2001; Venel, Mullen et al. 2001; Liu, Lyon et al. 2003). However, although these studies indicated that NIR spectroscopy is feasible and promising in the quality control of meat products, the results, especially on meat tenderness predictions, have been inconsistent, partially because NIR spectroscopy measures the overtones of fundamental molecular vibration modes which are often overlapped to yield broad bands that do not provide high resolution information about the biochemical landscape of the meat. Also, in many researches in which optical or ultrasonic measurements were investigated as quality control means, they were evaluated by their correlations to shear forces values (Beattie, Bell et al. 2004), which make their relevance in determining tenderness and/or chewiness even more questionable.

Raman spectroscopy is another alternative method that has a considerable number of advantages compared to other food analysis techniques (Colthup, Daly et al. 1964) (Beattie, Bell et al. 2004). It is a noninvasive spectroscopic technique providing in situ information about the composition and structure of proteins and lipids, which are main components of pork (Li-Chan 1996; Brondum, Byrne et al. 2000; Pedersen, Morel et al. 2003; Beattie, Bell et al. 2004; Olsen, Rukke et al. 2007; Herrero 2008; Herrero 2008). Raman spectroscopy is relatively insensitive to water and hence does not suffer from water interference, which is a severe problem in mid-IR spectroscopy like FT-IR, since foods commonly contain $\geqslant$75% water. In addition, it does not require any sample preparation and is non-destructive whilst at the same time providing detailed spectral information about the chemical composition of the sample.

Raman spectroscopy has been used to predict sensory quality of beef silverside (Beattie, Bell et al. 2004). A relatively good correlation between Raman data and sensory panel's ratings of acceptability of texture and degree of tenderness was reported. However, previous studies did not establish a working model for predicting tenderness and/or chewiness of meat samples(Beattie, Bell et al. 2004).

Support Vector Machine (SVM) belongs to a new generation of machine learning system based on recent advances in statistical learning theory (Steinwart and Christmann 2008) for classification or regression. It is an extension to nonlinear models of the generalized portrait algorithm developed by Vladimir Vapnik (Drucker, Burges et al. 1997). The SVM algorithm is based on the statistical learning theory and the Vapnik-Chervonenkis (VC) dimension introduced by Vladimir Vapnik and Alexey Chervonenkis (Vapnik 2000). It is particularly suitable to separate two distinguishable groups. In SVM, input data are viewed as two sets of vectors in an *n*-dimensional space, an SVM will construct a separating hyper plane in that space, one which maximizes the margin between the two data sets. To calculate the margin, two parallel hyper planes are constructed, one on each side of the separating hyper plane, which is "pushed up against" the two data sets. Intuitively, a good separation is achieved by the hyper plane that has the largest distance to the neighboring data points of both classes, since in general larger the margin, better the classification.

In this work, we developed a Raman spectrosensing method in conjunction with support vector machine modeling to predict the sensory tenderness and chewiness of fresh pork loins, with excellent accuracy (>82%) for selection of the pork samples with tenderness/chewiness values at the two extreme ends. Potentially the Raman method can serve as selection tools to quickly screen and separate high quality (very tender) and low quality (very tough) meat during the meat processing on a slaughterhouse floor.

MATERIALS AND METHODS

Animals and sampling

All animal procedures were approved by the Animal Care and Use Committee of Iowa State University. Gilts from the fifth generation of pigs in the Iowa State residual feed intake study (Cai, Casey et al. 2008) were utilized. This experiment utilized Select line gilts (n=80) and Control line gilts (n=89). The melanocortin-4 receptor genotype was sequenced in these animals using the method described by Kim et al (Kim, Larsen et al. 2000). Gilts were placed in finishing pens at 98± 5 d of age and 39.7±5.7 kg and fed a diet formulated to meet or exceed nutrient requirements (NRC, 1998). Individual feed intake was recorded with electronic 1-space feeders (FIRE, Osborne Industries Inc., Osborne KS) as described by Cai et al(Cai, Casey et al. 2008). Gilts were slaughtered in a commercial facility in three groups over an 8 week period (July-September). Slaughter weight was approximately 114 kg. Pigs were rendered unconscious through carbon dioxide stunning. The boneless loins were removed from the carcass at 24 h postmortem, vacuum packaged, and were transported to the ISU Meat Laboratory on the same day. Boneless center loins (2 d postmortem) were separated into 2.54 cm chops at the ISU Meat Laboratory. Loin chops that were to be used for sensory and star probe analysis were vacuum packaged and held for 7 to 10 d postmortem at 4˚C. Samples to be used for Raman measurement were vacuum packaged and held at 4˚C until they were frozen at either 2 d or 7 d postmortem.

Sensory tenderness, star probe and sensory chewiness Analysis

Star probe values and sensory quality scores were determined on cooked pork loin chops. Chops aged 7-10 d postmortem were cooked on clamshell grills to an internal temperature of 70 ˚C. The temperature of each chop was monitored individually using thermocouples (Omega Engineering, Inc. Stamford, CT). The chops were cooled to room temperature prior to analysis. Star probe is an instrumental measure of texture that determines the amount of force necessary to compress the sample to 80% of its height(Lonergan, Stalder et al. 2007). A circular, five-pointed star probe that measures 9 mm in diameter with 6 mm between each point was attached to an Instron Universal Testing Machine (Model 5566, Instron, Norwood, MA). Each chop was punctured at a crosshead speed of 3.3 mm/second. Each chop was punctured three times and the average of the three values determined the overall value(Lonergan and Prusa 2002). A trained sensory panel (n=4) evaluated sensory traits on loin chops aged 7-10 d postmortem. The chops were cooked on clamshell grills to an internal temperature of 70˚C. Four cubes were cut from the center of the chop and each panelist evaluated the samples for juiciness, tenderness, chewiness, pork flavor, and off-flavor. An unanchored scale was used with a term that represented a low degree of juiciness, tenderness, chewiness, pork flavor, and off-flavor on the left and a term that represented a high degree on the right side of the scale.

Sample preparation and Raman measurements

Two small pieces with 3 mm thickness of each pork sample were cut and stored in -20ºC individually. They were fully thawed at ambient temperature before measurement. Raman measurements were performed using a DXR Dispersive Raman Microscope (Thermo Scientific, Inc., Madison, WI) with 780nm, 14 mW excitation laser at ambient temperature. Raman spectra were collected with 2s exposure time from 400 and to 2000 cm^{-1} at a resolution of 1 cm^{-1}. The pork samples were placed directly on glass slides at the focus of the laser beam with no pretreatment.

Spectral data processing

All spectra were automatic baseline corrected and smoothed to reduce the baseline variability at the region between 400cm^{-1} to 2000cm^{-1} and normalized using Omnic professional Software Suite (Thermo Scientific, Inc., Madison, WI). The first derivative and second derivative spectra were calculated from the normalized spectra.

When Raman spectral data are used to construct chemometric models to classify and/or differentiate pork samples with distinct properties, the most important spectral signatures are the fingerprinting Raman peaks that represent the biochemical landscape of the pork sample. Raman peaks are represented by their wavenumber (Raman shift) and intensity. The peak intensities are dependent on many factors that may vary from sample to sample (i.e., sample size, exposure time, etc.), but their Raman shift remain identical as long as the molecular makeup is the same. Therefore, in this study we developed a binary barcode to eliminate variations in the spectral data due to peak intensities, and highlight the unique Raman shift fingerprints of each sample. The binary barcode approach was originally proposed by Ziegler and coworkers (Martens and Naes 1992) to differentiae microorganisms based on their Raman spectroscopic signatures, in this study a similar approach was developed to improve the classification accuracy of pork loins.

The binary barcodes were generated based on the second derivative spectra in the 400cm^{-1} to 2000cm^{-1} range. A binary value (0 or 1) was assigned to each second derivative spectral data point primarily based on the sigh of the second derivative, i.e., 1 for upward curvature (positive second derivatives), and 0 for downward curvature (negative second derivatives). Furthermore, A threshold for zero was set at 6% of the maximum value of the second derivative for positive second derivative readings (for all value larger than the threshold, 1 was retained; otherwise it was switched to 0). This threshold helps discriminate against residual noise components. Contribution to the measured spectra from low level background noises was thus removed by assigning 0 to it. Remaining 1s represent contributions to the measured spectra from meat components. The threshold value (6%) was determined experimentally by finding the barcodes that provided the best prediction for the sensory attributes.

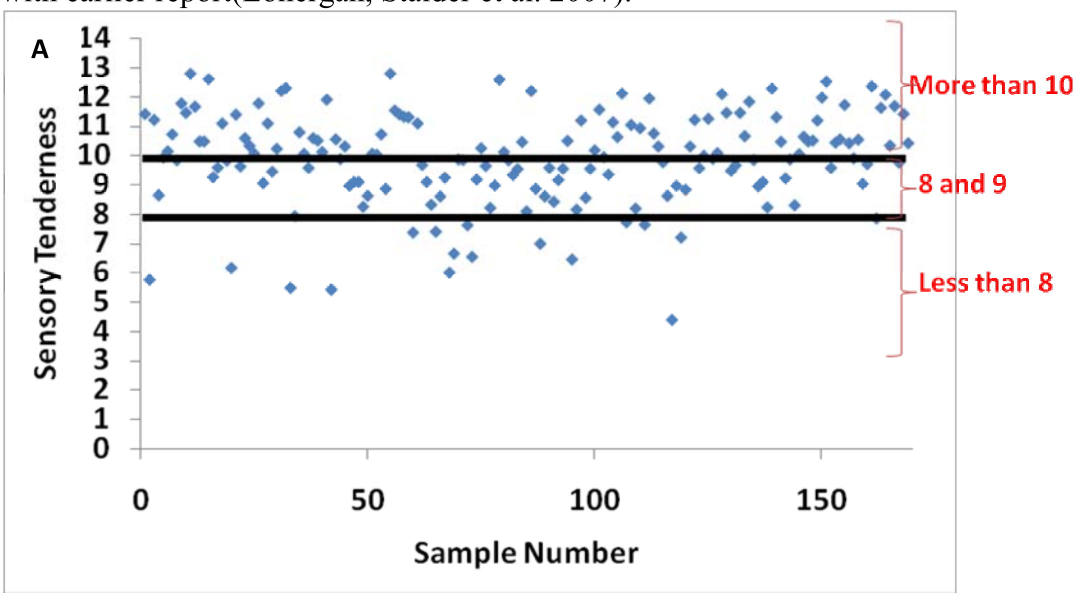
Chemometric analysis

The multivariate data analysis was carried out using Support Vector Machine (Steinwart and Christmann 2008) implemented with Matlab SVM toolbox. Partial Least Square (PLS) algorithm was used to further compress the data sets (the binary barcodes) and generated inputs for the SVM model. Our main goal is to predict sensory attributes (i.e., tenderness and chewiness) that are at the two ends of the panel evaluation spectrum. The 169 pork loin samples were divided into 3 groups according to the value of specific sensory attributes and/or star probe values. Then, one calibration set and one test set were set in such a way that both the calibration set and the test set showed approximately the same distribution of one specific variable. Different calibration samples were chosen randomly to calculate the average classification accuracy (over 10 random sampling). Chemometric analysis was conducted using both WinDas ((Wiley & Sons, Chichester, UK, 1998 version) and Matlab (The mathworks, Natick, MA) software.

RESULTS AND DISCUSSION

Sensory tenderness, star probe and sensory chewiness

Values of sensory tenderness, chewiness and star probe vary significantly between samples, as shown in Fig. 1. For tenderness, on a scale of 0-15, the 169 samples were found to be between 4 and 13, with higher scores being superior quality. For chewiness, on a scale of 0-10, the 169 samples were found to be between 1 and 9, with lower scores being superior quality. Star probe values distributed between 3.0 and 8.0, they were negatively correlated to sensory tenderness scores, which was in agreement with earlier report(Lonergan, Stalder et al. 2007).



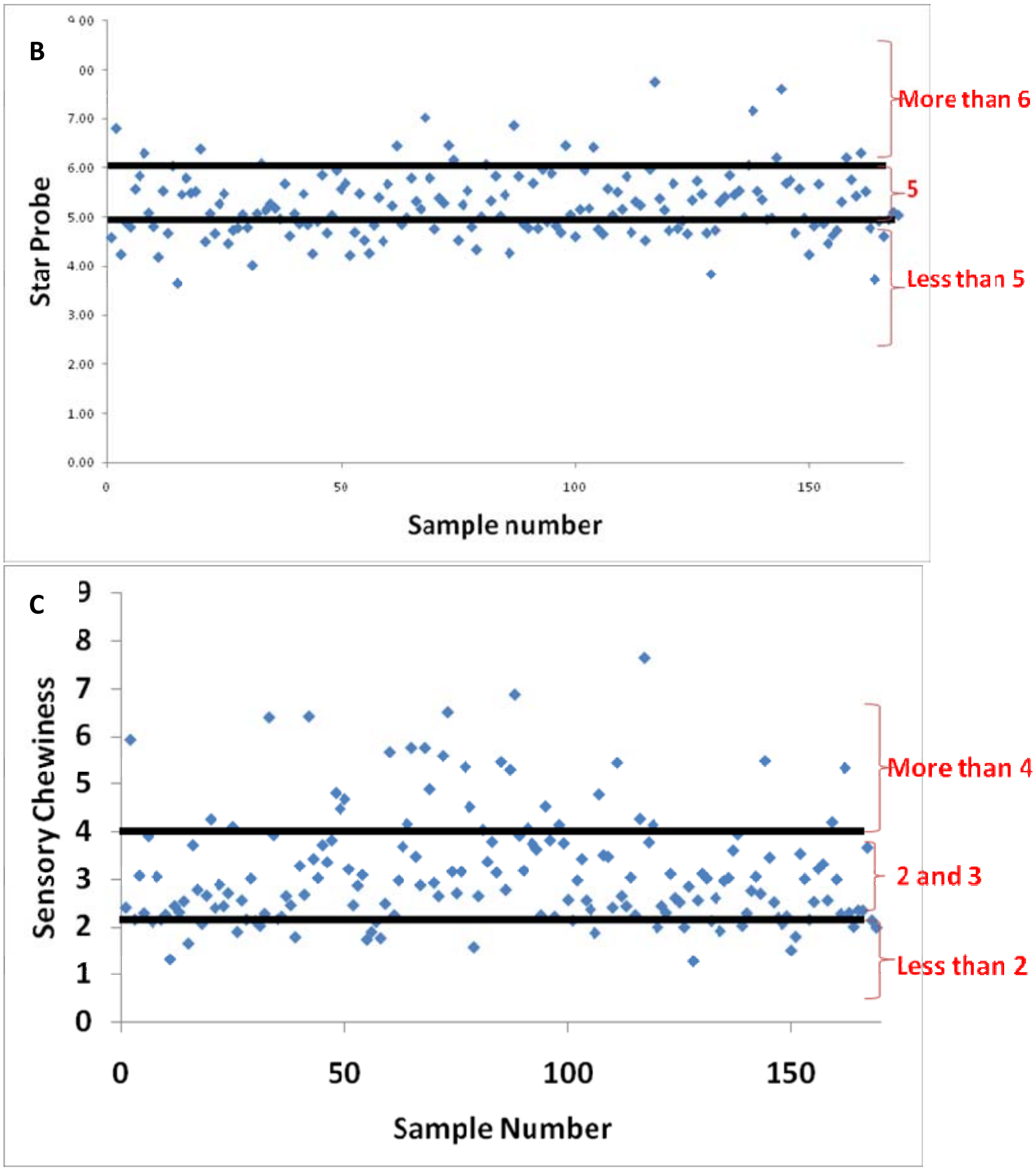


Fig 1. Sensory tenderness (A), star probe (B) and sensory chewiness (C) for 169 pork samples

Since our primary goal is to correctly predict pork samples that fall into the two extreme ends of their sensory attributes, we divided the samples into three groups: high quality (tenderness score >10, chewiness score <2), medium quality (10 > tenderness score > 8, 4 > chewiness score > 2) and poor quality (tenderness score < 8, chewiness score > 4).

Raman spectroscopic analysis

Typical Raman spectra of pork samples in the 400-2000cm^{-1} region are shown in Fig2. Baseline correction, smooth and normalization from original spectra were applied to remove background noises. The assignments of the corresponding Raman bands are listed in Table 1. The wavenumber and intensity changes in the Raman bands were indicative of changes in the secondary structure and variations in local environments of meat proteins, which in turn determine the characteristics/properties of the meat. For instance, the Raman band centered near 1653cm^{-1} (Table 1), represents amide I vibration mode which is a indictor of the overall concentration of proteins(Herrero 2008).

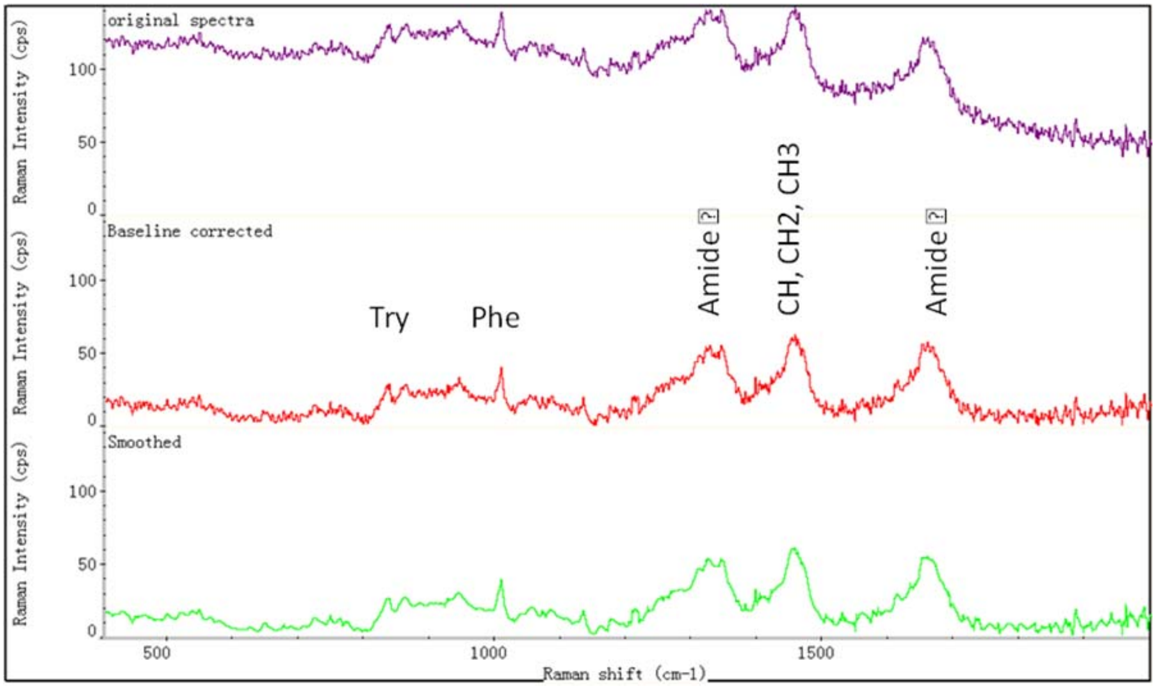


Fig. 2 Baseline correction and smooth of the typical Raman spectrum of pork loins

Table 1 Important Raman bands in meat

Raman shift (cm^{-1})	Type of vibration
3016	Asymmetric =C–H stretch
2935	Asymmetric CH_2 stretch
2900	Symmetric CH_3 stretch
2854	Symmetric CH_2 stretch
1750	C=O stretch
1653	cis C=C stretch/amide I
1521	In-phase C=C stretch (carotenoids)
1441	CH_2 scissoring
1302	CH_2 twist
1266	Symmetric =C–H rock
1159	Polyene chain C–C stretch (carotenoids)
1122/1081/1066	C–C/C–N/C–O stretch
1004	Aromatic ring breathing (Phenylalanine)
974	=C–H out-of-plane deformation
932/866	C–C/C–O stretch

In spectroscopic data processing, first and second derivatives are routinely calculated to remove slowly varying background noises which otherwise would contribute non-essential variances to the subsequent statistical analysis. First derivative spectra avoid contributions resulting from fluctuations in spectral background, but are still sensitive to Raman vibration intensity fluctuations. Second derivative spectra similarly minimize background variability and tend to further reduce sensitivity to intensity fluctuations. Furthermore, the signs of the second derivatives, indicating the locations of peaks and valleys, are found to be extremely robust identification features with minimal variability in replicated measurements. The binary barcodes (with a 6% threshold) calculated from these signs of second derivatives further eliminated signal fluctuations due to all the sources of intensity variations, as shown in Fig 3.

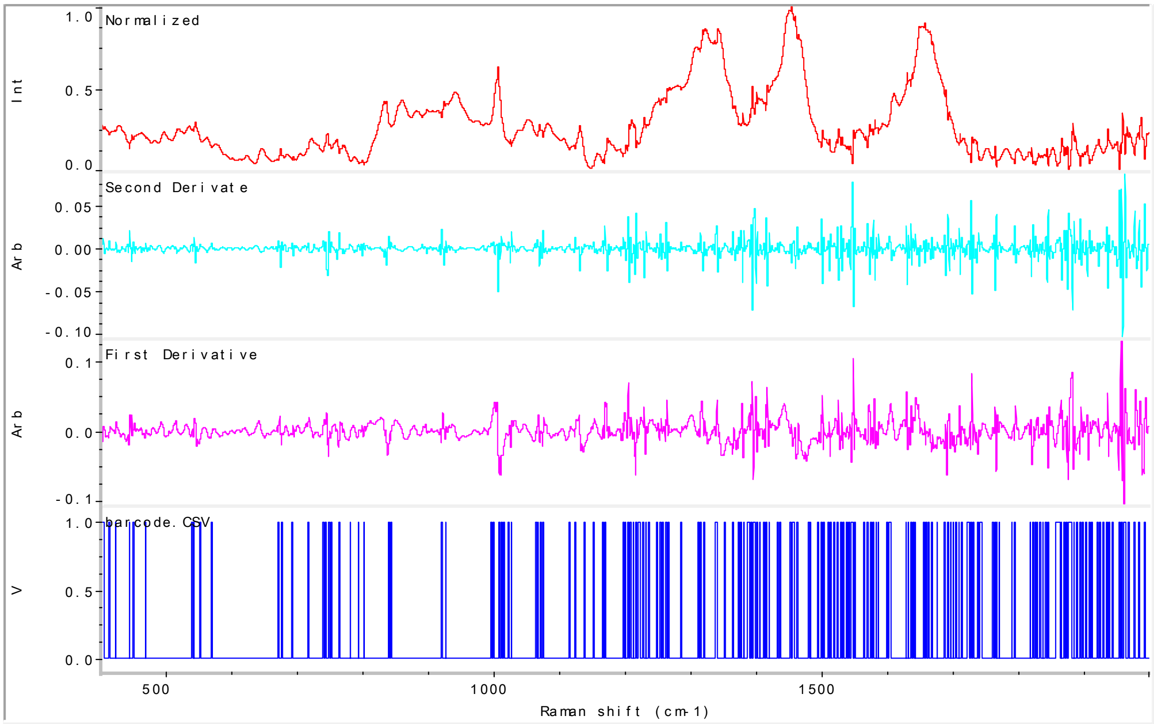


Fig 3 First derivative spectrum, second derivative spectrum and binary barcodes spectrum of a typical pork loin sample.

The selection of a threshold was determined through investigation of the optimal threshold value that would yield the best classification accuracy. The results are shown in fig. 4. Threshold values of 0-24% of the maximum second derivatives were investigated, and 6% was identified as the optimal value to retain the most information that yields the best classification results. It was used throughout the study.

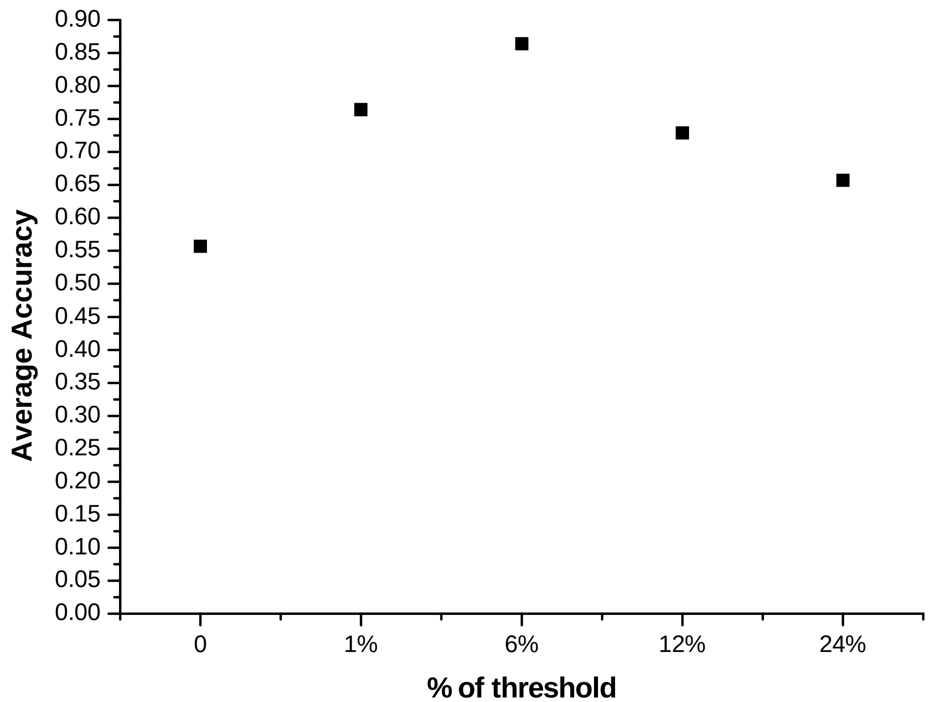


Fig. 4 Determination of the threshold for binary barcode spectral processing

Classification of pork loins by sensory tenderness and sensory chewiness

Firstly it was investigated that whether or not the pork loins could be classified into three distinguishable quality groups (good, medium, poor) as defined by their tenderness or chewiness values based on their Raman spectroscopic characteristics. As shown in Fig.5, using the binary barcodes for each pork samples, with canonical variant analysis, a classification based on tenderness (Fig.5a) and chewiness (Fig.5b) was achieved that demonstrated three well-separated groups for each quality category. The successful classification shows that the Raman spectroscopic binary barcodes for different pork samples are uniquely correlated to their sensory attributes.

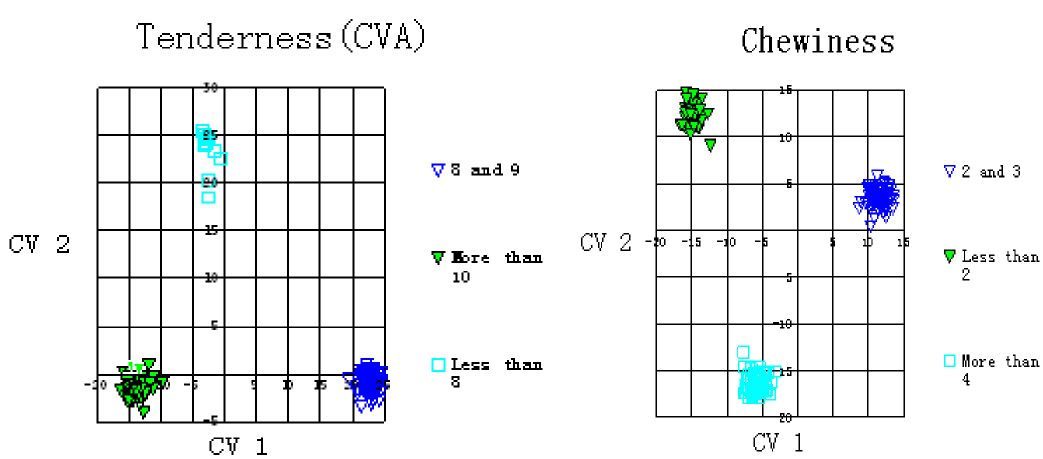
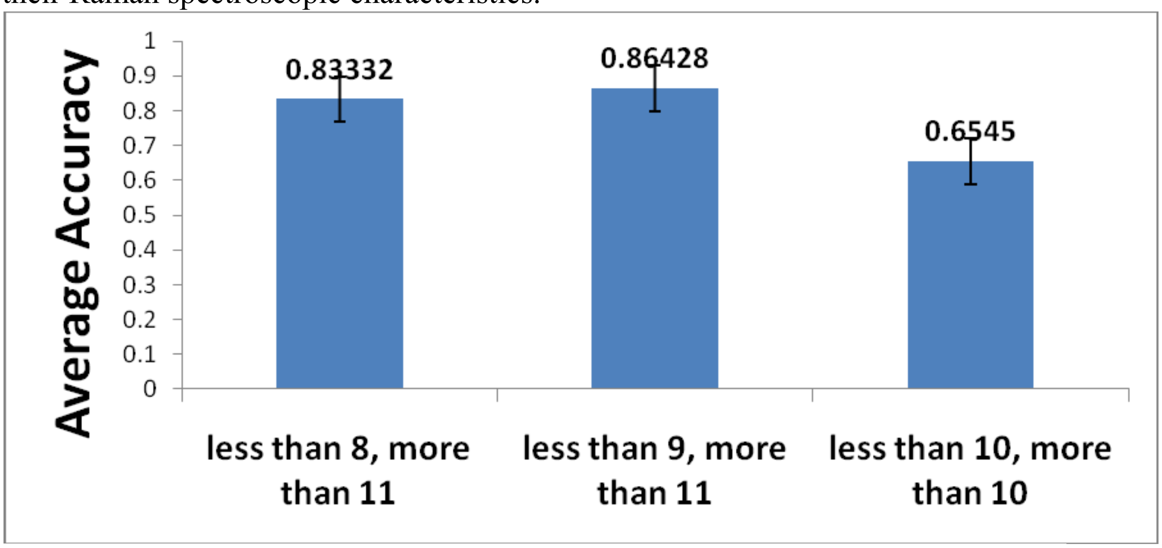


Fig. 5 Classification of pork loins into three quality categories based on their Raman spectroscopic barcodes and sensory panel classifications
(a. Left panel: for tenderness; b. right panel, for chewiness)

Furthermore, the PLS generated clusters were employed in a support vector machine (SVM) discriminant model to classify unknown pork loin samples into different quality categories based on their Raman spectroscopic binary barcodes. The results are shown in Fig 6. For each test, we randomly selected 100 spectra of known pork samples to construct a training set, and then spectra from 20 randomly chosen, unclassified samples were used for testing. The process was repeated for 5 times and the average classification accuracy was calculated. The classification accuracy for correct predicting a sample that belongs to an extreme category (good vs. poor) is about 83%-86%. Adjusting the boundaries on the quality categories (poor from <8 to <9) did not significantly change the prediction accuracy. However, if a simple separation line was set (tenderness score = 10), the prediction accuracy diminished significantly. Apparently, pork samples that belong to the medium quality category are difficult to predict based on their Raman spectroscopic characteristics.



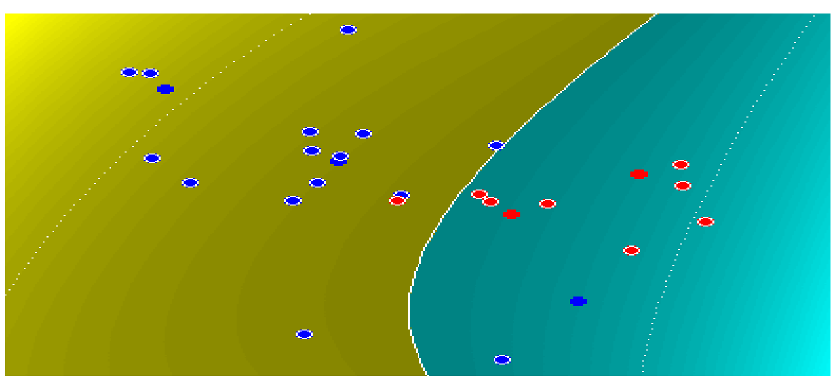
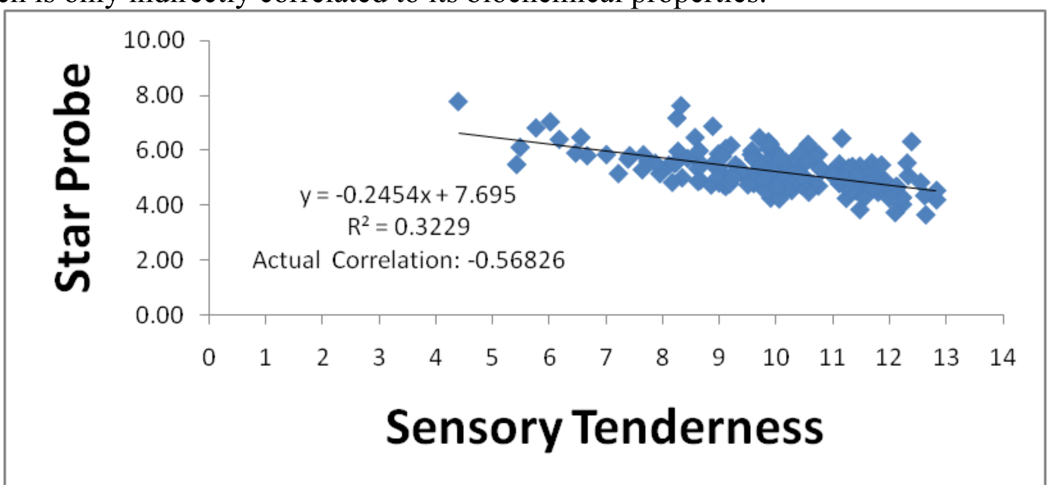


Fig. 6 Prediction of classifying pork samples into different tenderness grades based on their Raman spectroscopic barcodes.
(a: top panel, prediction accuracy when different tenderness grades were used; b: bottom panel, SVM prediction results to classify samples into good (blue, tenderness>11) and

poor (red, tenderness < 9), it should be noticed that this is a 2-D projection of a 10-D hyper plane separation, the "circled" dots represent the correct predictions)

As a comparison, correlation between star probe values and sensory tenderness of the pork samples was shown in Fig. 7a. The correlation was not very good, with a correlation coefficient equals to -0.56826, suggesting once again that mechanical measurement does not always correlate well with sensory tenderness. Furthermore, the prediction accuracy for star probe categories (in parallel with the tenderness categories) was worse than that for the actual sensory tenderness. Since tenderness is primarily determined by the biochemical characteristics of the meat, it is reasonable that Raman spectroscensing, which measures the biochemical landscape of the meat, is indeed a better tool to predict sensory tenderness than to predict mechanical properties of the meat, which is only indirectly correlated to its biochemical properties.



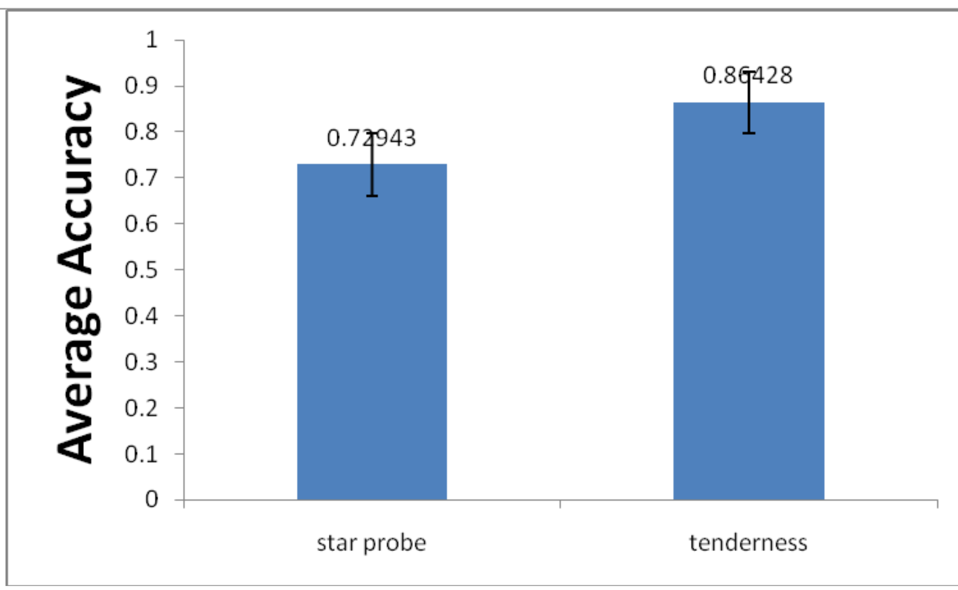
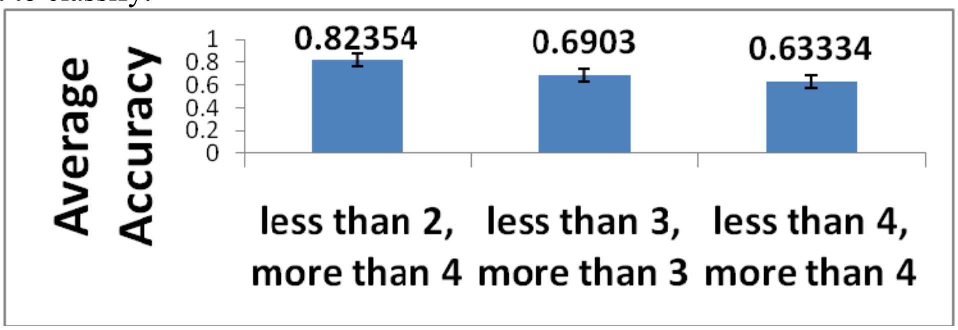


Fig. 7 Comparison between mechanical measurements and Raman spectrosensing in determining sensory tenderness

(a. Correlations between star probe value and sensory tenderness; B. Prediction accuracy for classification of sensory tenderness and for star probe values)

Classification for sensory chewiness was also conducted using similar approaches as for sensory tenderness using Raman spectroscopic binary barcodes for pork loin samples. The results are shown in fig. 8. Once again, the prediction accuracy for classfying pork loin samples at the extremes of the attribute spectrum (good, chewiness score <2, and poor, chewiness score > 4) was excellent, 82% of the samples in these catogeries were correctly classfied. The discriminant model did not work as well for pork samples with medium grade of chewiness. If the classification criterion was set to be separate the samples only into two catogeries with the boundary at chewiness score of 3 and 4, the prediction accuracy dropped to ~70% and 63%, respectively. Similar to the case of sensory tenderness, pork samples with medium levels of chewiness are the most difficult to classify.



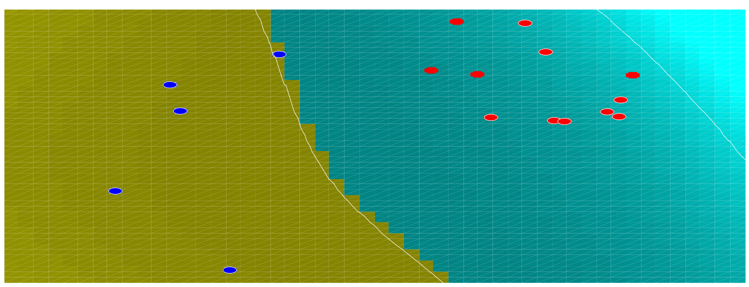


Fig. 8 Prediction of classifying pork samples into different chewiness grades based on their Raman spectroscopic barcodes.
(a: top panel, prediction accuracy when different tenderness grades were used; b: bottom panel, SVM prediction results to classify samples into good (blue, chewiness < 2) and poor (red, chewiness >4), it should be noticed that this is a 2-D projection of a 10-D hyperplane separation, the "circled" dots represent the correct predictions)

CONCLUSION:

In this report, a Raman spectrosensing method was developed to differentiate and classify pork loins into grades by sensory tenderness and chewiness. The method was demonstrated to yield good performance in identifying pork loins that belong to extreme categories of their sensory quality (i.e., really good and really poor ones). It is shown that Raman spectroscopy, in combination with performance-enhancing data processing and multivariate statistical discriminant modeling, has the potential to become a rapid on-line screening tool for the pork producers to quickly select meats with superior quality and/or poor quality to better serve customers.

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